of water and chromatographed under the same conditions as for 2 and 4. The desired fractions were collected, treated again with cation exchange resin, and lyophilized to give 14 mg (36%) of the desired free acid dinucleotide 3 as a white fluffy solid. The purity and identity of the product was confirmed by HPLC, MS, and ^{1}H NMR analyses.

IMP Dehydrogenase Inhibition. Kinetics of inhibition of a partially purified preparation of IMPD was examined as described previously[13] by using 3 concentrations of inhibitor and 6 concentrations of the substrates NAD or IMP (0.25, 0.5, 1, 2, 4, and 6 × K_m). Results were subjected to double-reciprocal analysis using a least-squares computer program developed in this laboratory.

Cytotoxicity. These studies were performed as described previously for the parent nucleoside TR with use of P388 cells in log phase growth.[19]

Degradation of TAD and TAD Phosphonates. TAD and its phosphonate analogues were subjected to hydrolysis by either partially purified TAD phosphodiesterase or snake venom phosphodiesterase at a substrate concentration of 7.5 mM in 0.03 M Tris-$MgCl_2$ buffer, pH 7.6. Quantitation of AMP or TRMP by HPLC was taken as a measure of dinucleotide degradation after a 30-min incubation period.

Tiazofurin-Resistant P388/R Line. The P388 resistant cell line used in this study was developed by exposing P388 cells to gradually increasing concentrations of TR until the population could grow almost normally in the presence of a 1 mM concentration of the drug; the doubling time was 17 h as opposed to 15 h for the parent line. To compare the metabolism of TR in these two lines, they were incubated with [^{14}C]-labeled drug for 5 h after which the cells were washed, and the pellets were extracted with 10% TCA and neutralized with tri-*n*-octylamine in freon. Using 90% methanol as solvent, paper chromatography (Whatman 3MM) was carried out on the resultant extracts. Spots coincident with TAD were then cut out, eluted with water, and counted for radioactivity. TAD levels in the sensitive and resistant cells were 14.3 and 0.98 pmol/10^6 cells, respectively.

Influence of β-Methylene-TAD (3) on the Guanine Pools of P388/S Cells and TR-Resistant P388/R Mutants. Logarithmically growing P388/S or P388/R cells were incubated with the indicated concentration of β-methylene-TAD or TR for 18 h (Table III). The cells were then washed once with cold normal saline, extracted with cold 10% TCA (0.4 mL), and finally neutralized with an equal volume of tri-*n*-octylamine in freon. The resultant extracts were then analyzed by HPLC utilizing a radial compression column of Partisil-10 SAX resin (Waters Co., Milford, MA) pre-equilibrated and isocratically eluted with 0.03 M ammonium phosphate, pH 3.5, for 10 min. A 30-min gradient to 0.6 M ammonium phosphate, pH 3.8, followed, and the column was washed with the 0.6 M buffer for 10 min. In sensitive control cells, the levels of GMP, GDP, and GTP were 0.14, 0.20, and 0.39 nmol/10^6 cells, respectively. In resistant control cells the same nucleotides were 0.18, 0.28, and 1.0 nmol/10^6 cells, respectively.

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Registry No. 1, 83285-83-0; 2, 102977-54-8; 3, 102977-57-1; 4, 102977-55-9; 5b, 60084-11-9; *cis*-6b, 102977-49-1; *trans*-6b, 102977-50-4; 7b, 102977-51-5; 8b, 102977-52-6; 9b, 102977-53-7; 10a, 22257-15-4; 11, 7331-13-7; 13, 3768-14-7; 14, 102977-56-0; TRMP, 83161-83-5; IMP dehydrogenase, 9028-93-7; diphenyl triphenylphosphoranylidene methylphosphonate, 22400-41-5.

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Synthesis of 11*H*-Pyridocarbazoles and Derivatives. Comparison of Their DNA Binding and Antitumor Activity with Those of 6*H*- and 7*H*-Pyridocarbazoles

Elie Lescot,*† Gabriel Muzard,† Judith Markovits,† Joël Belleney,‡ Bernard P. Roques,‡ and Jean-Bernard Le Pecq†

Laboratoire de Pharmacologie Moléculaire, LA 147 (CNRS), U 140 (INSERM), Institut Gustave-Roussy, 94800 Villejuif, France, and Département de Chimie Organique, ERA 613 (CNRS), U 266 (INSERM), UER des Sciences Pharmaceutiques et Biologiques, 75006 Paris, France. *Received July 22, 1985*

The 8-methoxy- and 8-hydroxy-11*H*-pyrido[2,3-*a*]-, -[3,4-*a*]-, -[4,3-*a*]-, and [3,2-*a*]carbazoles were synthetized as potential DNA intercalating antitumor drugs. The structure of these compounds was confirmed by ^{1}H NMR study including NOE experiments. The DNA binding properties of substituted and unsubstituted (8-*H*) heterocycles were determined by using their hydrochlorides or methiodides. These derivatives are able to bind to DNA with an affinity varying from 2.0 × 10^4 to 1.0 × 10^6 M^{-1}, but most of them are unable to intercalate in contrast with the behavior of 6*H*- and 7*H*-pyridocarbazole analogues. The cytotoxicity of 11*H*-pyridocarbazoles, measured on L1210 cells in vitro, is much lower than those of 6*H*- and 7*H*-pyridocarbazole analogues.

The antitumor drug 2-methyl-9-hydroxyellipticinium acetate has recently been introduced in the treatment of breast cancer.[1] It is a DNA intercalating compound derived from the ellipticine alkaloid,[2] which belongs to the 6*H*-pyridocarbazole series (Chart I). 9-Methoxyellipticine also exhibits antitumor properties.[3] The antitumor activity of these derivatives is in part related to their high DNA binding affinity.[2] The size and the shape of the 6*H*-pyridocarbazole ring lead to an almost perfect overlapping of the aromatic ring with that of a DNA base pair.[4] Therefore, the pyridocarbazole ring appears as an appropriate skeleton to design DNA intercalating drugs. Accordingly, various 7*H*-pyridocarbazole derivatives have recently been synthesized[5] and have led to antitumor drugs in the series of bifunctional intercalators.[6] Furthermore, the antitumor potency of these heterocycles seems to be enhanced by the introduction of a hydroxy or a methoxy group in the para position with respect to the indolic NH

† Institut Gustave-Roussy.
‡ UER des Sciences Pharmaceutiques et Biologiques.

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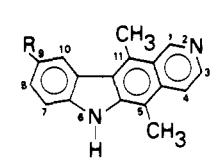
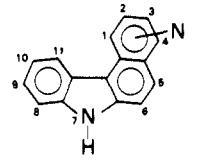
Chart I

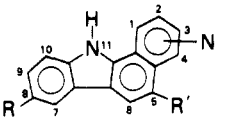


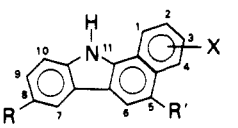
6H-PYRIDOCARBAZOLES

R=H ; Ellipticine
R=OCH$_3$; 9-Methoxyellipticine
R=OH ; 9-Hydroxyellipticine



7H-PYRIDOCARBAZOLES





11H-PYRIDOCARBAZOLES

Compound	R	R'	Compound	X	R	R'
4 ; 1-N	H	H	17	1-NH,Cl$^-$	H	H
5 ; 1-N	OCH$_3$	H	18	1-NH,Cl$^-$	OCH$_3$	H
6 ; 1-N	OH	H	19	1-NH,Cl$^-$	OH	H
7 ; 3-N	H	H	20	3-NCH$_3$,I$^-$	H	H
8 ; 3-N	OCH$_3$	H	21	3-NCH$_3$,I$^-$	OCH$_3$	H
9 ; 4-N	H	H	22	4-NCH$_3$,I$^-$	H	H
10 ; 4-N	OCH$_3$	H	23	4-NCH$_3$,I$^-$	OCH$_3$	H
11 ; 4-N	OH	H	24	4-NCH$_3$,I$^-$	OH	H
12 ; 2-N	H	CH$_3$	25	2-NCH$_3$,I$^-$	H	CH$_3$
13 ; 2-N	OCH$_3$	CH$_3$	26	2-NCH$_3$,I$^-$	OCH$_3$	CH$_3$
14 ; 2-N	OH	CH$_3$	27	2-NCH$_3$,I$^-$	OH	CH$_3$

(position 9 in the 6*H*-pyridocarbazoles and position 10 in the 7*H*-pyridocarbazoles series).

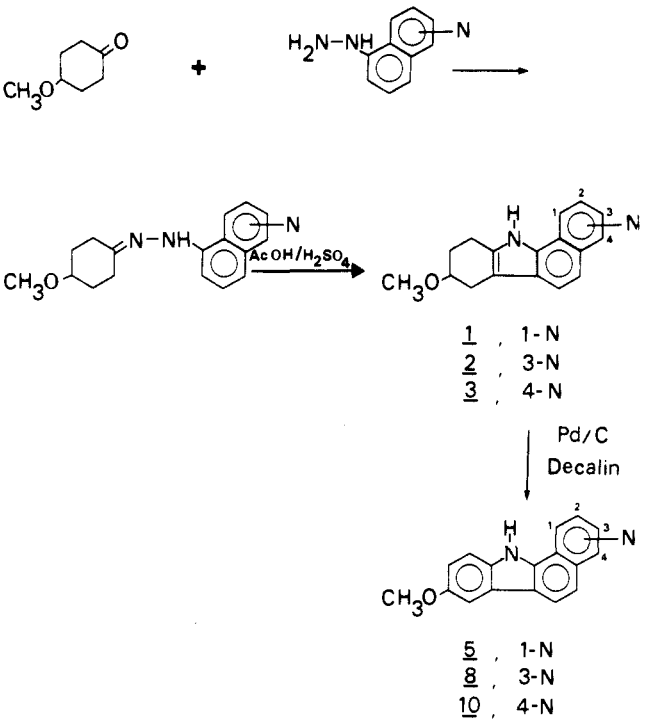
In the 6*H*-pyridocarbazoles, the nitrogen atom of the pyridinic ring can occupy two different positions leading to two isomers, while in the 7*H*-pyridocarbazole series, four isomers can be obtained. However, only 9-methoxy- or 9-hydroxy-6*H*-pyrido[4,3-*b*]carbazole (ellipticines) and 10-methoxy-7*H*-pyrido[4,3-*c*]carbazole elicited potent antitumor activity.[2,3,5]

It was therefore of interest to study the corresponding derivatives in the series of 11*H*-pyridocarbazoles. In this paper, we report the synthesis of isomeric 8-methoxy (**5**, **8**, **10**, **13**)- and 8-hydroxy (**6**, **11**, **14**) -11*H*-pyridocarbazoles and the DNA binding properties of their hydrochlorides (**17**–**19**) or methiodides (**20**–**27**) (Chart I). Moreover, the antitumor activity of these compounds was evaluated on L1210 cell in vitro in parallel with the corresponding derivatives of the 6*H*- and 7*H*-pyridocarbazole series including the antitumor drug 2-methyl-9-hydroxyellipticinium acetate.

Chemistry. Following the method already described[7-11] for the unsubstituted 11*H*-pyridocarbazoles **4**, **7**, and **9**, compounds **5**, **8**, and **10** were obtained by using 4-methoxycyclohexanone as starting material. However, attempts to aromatize the 8-methoxy-7,8,9,10-tetrahydro-11*H*-pyridocarbazoles **1**–**3**, precursors of **5**, **8**, and **10**, on heating at 320 °C with 10% palladium on activated charcoal or with selenium[10] gave the corresponding demethoxylated derivatives **4**, **7**, and **9**. Compounds **5**, **8**, and **10** were finally obtained by boiling the corresponding methoxylated tetrahydro derivatives in decahydronaphthalene with 10% palladium on activated charcoal (Scheme I). The synthesis of **12** and **13** followed the route adopted by Joule et al.[12] for the hemisynthesis of subincanine (Scheme II).

Demethylation of **5**, **10**, and **13** was performed in boiling 48% aqueous hydrobromic acid giving the hydroxylated compounds **6**, **11**, and **14**, respectively.

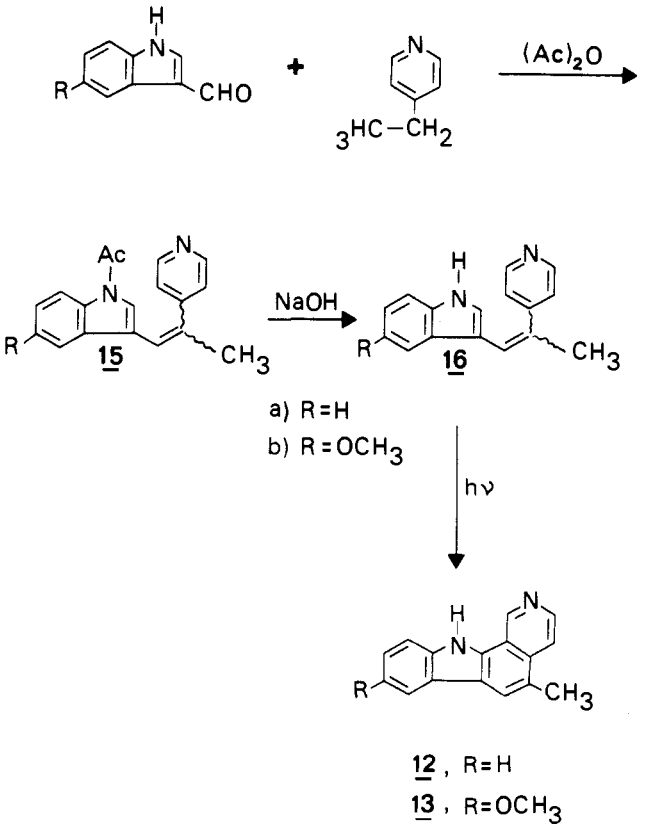
Scheme I



1, 1-N
2, 3-N
3, 4-N

5, 1-N
8, 3-N
10, 4-N

Scheme II



a) R=H
b) R=OCH$_3$

12, R=H
13, R=OCH$_3$

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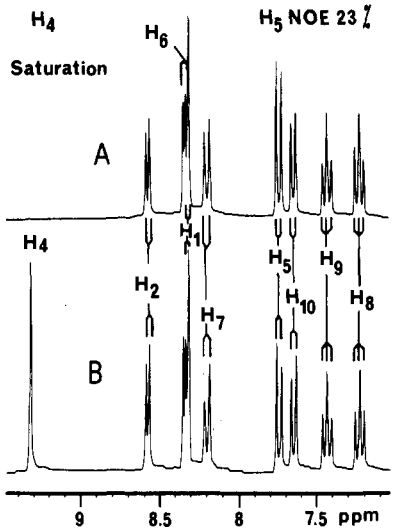

Figure 1. ^{1}H NMR spectra of 11*H*-pyrido[4,3-*a*]carbazole **7**: (A) saturation of H-4; (B) normal spectrum.

Compounds **7–14** were quaternized with methyl iodide, while **4–6** failed to react. This lack of reactivity is likely due to the steric hindrance between the pyridinic nitrogen at position 1 and the NH of the carbazole ring. Their hydrochlorides, **17–19**, were prepared instead.

The structure of compounds **4–14** was confirmed by ^{1}H NMR study, including nuclear Overhauser effect (NOE) experiments. Compound **7**, 11*H*-pyrido[4,3-*a*]carbazole, is taken as an example (Figure 1). The singlet that appears at 9.36 ppm is assigned, without ambiguity, to the proton H-4. Saturation of this signal results in a 23% (NOE) increase of the doublet at 7.74 ppm, which corresponds to the proton H-5. Irradiation of H-5 (without spin decoupling) produces in turn a 24% Overhauser increase for H-4 and a 10% increase for H-6 (8.33 ppm). Irradiation of H-6 (without spin decoupling) produces a 13% enhancement for H-5 and 21% for H-7 (8.19 ppm). Finally, NOE of 23% and 19% are observed for H-6 and H-8, respectively, when H-7 is irradiated. The proton H-8 (7.22 ppm) being thus located, H-9 (7.43 ppm) and H-10 (7.65 ppm) are respectively identified by irradiation with spin decoupling. Identification of H-1 (8.32 ppm) and H-2 (8.57 ppm) is achieved by irradiation of H-5 provoking the disappearance of the zigzag-type coupling between H-1 and H-5 and a subsequent narrowing of the H-1 signal (0.5 Hz). Likewise, a narrowing of signals H-6 and H-7 is observed after exchange of the carbazole ring NH with heavy water.

When 11*H*-pyridocarbazoles are substituted at position 8 (compounds **5**, **6**, **8**, **10**, **11**, **13**, **14**), ^3J and ^4J couplings between H-9, H-10 (9 Hz) and H-9, H-7 (3 Hz) are observed. To avoid misinterpretation between H-5 and H-6, proton H-7 was irradiated yielding a NOE enhancement for H-6 (15–18%), and irradiation of H-6 produces a 15% and 10% increase for H-7 and H-5, respectively.

Two singlets are observed for compounds **12–14** corresponding to H-1 and H-6. To identify H-6, the methyl group at position 5, for compound **13**, was irradiated (without spin decoupling) producing thus a NOE of 23% for H-6 (8.21 ppm) and 17% for H-4 (7.82 ppm).

Interaction with DNA. The interaction of 11*H*-pyridocarbazoles and derivatives with calf thymus DNA was studied with the quaternary salts **20–27** and with the hydrochlorides **17–19** (Table I). DNA affinity constants (K_{ap}) were determined by competition with ethidium bromide or with ethidium dimer.[13,14] In the case of compound **25**, which was much more fluorescent when bound to DNA than when free in solution, the DNA binding affinity was directly measured by fluorescence titration. As shown in Table I, the 11*H*-pyridocarbazoles and derivatives that were studied display DNA binding affinities which are close to those found for 6*H*- and 7*H*-pyridocarbazole compounds.[2,5]

Table I. DNA Binding Parameters

compd	K_{ap} pH 5,[a,b] M^{-1}	slope[c]	$\phi°$ [d]
2-methylellipticinium acetate	2.3×10^5	2.04	13.0 (14.2)
2-methyl-9-hydroxyellipticinium acetate	1.3×10^6	2.32	(21.6)
2-methyl-10-methoxy-7*H*-pyrido[4,3-*c*]-carbazolium iodide[e]	3.0×10^5	2.30	(12.0)
2-methyl-10-hydroxy-7*H*-pyrido[4,3-*c*]-carbazolium iodide[f]	1.8×10^5	2.50	(14.5)
17	1.0×10^5	0	1.0
18	1.0×10^5		
19	2.0×10^4	0	
20	2.0×10^4	2.83	6.7
21	2.5×10^4	0.56	
22	1.0×10^4	2.45	14.0
23	6.0×10^4	0.23	4.4
24	1.0×10^5	0	
25	1.0×10^5	0.77	9.1
26	1.0×10^5	1.28	9.4
27	1.0×10^6	0.05	1.0

[a] K_{ap} value for compound **25** is determined by direct fluorometric titration. [b] K_{ap} value for compound **27** is estimated by competition experiments with ethidium dimer (ref 14). [c] The slope, n, of the function $\log [\eta]/[\eta]_0$ vs. $\log (1 + 2r)$ was determined, where $[\eta]$ and $[\eta]_0$ are the intrinsic viscosities of sonicated DNA in the presence and absence of drug and r is bound drug per nucleotide. Compound **18** precipitated in the conditions of viscometric measurements. [d] $\phi°$ is referred to as the DNA unwinding angle caused by the drug binding. Values in parentheses were determined by the viscosimetric method (ref 16). [e] D. Pelaprat, Thèse Doctorat d'Etat, Université Pierre et Marie Curie, Paris (June 1981). [f] Reference 5.

The ability of 11*H*-pyridocarbazoles to intercalate into DNA was studied by measuring the length increase of short DNA segments after drug binding. This parameter is determined from viscosimetric measurements as already described.[15,16] The slope, n, of the function $\log [\eta]/[\eta]_0$ vs. $\log (1 + 2r)$ was determined ($[\eta]$ and $[\eta]_0$ are the intrinsic viscosities of sonicated DNA in the presence and absence of ligand; r is bound ligand per nucleotide). Results are shown in Table I. The value of this slope is included between 2.4 and 3.0 when DNA intercalation takes place.[15]

Keller[17] has shown that the unwinding angle of a DNA helix resulting from drug interaction can be directly determined by following the change in the electrophoretic migration of superhelical DNA in agarose gel. In this method, the mean number, $\bar{\tau}$, of superhelical turns of circular DNA samples relaxed by topoisomerase I in the presence of various drug concentrations is measured. In that case, $\bar{\tau} = (\phi/360) \times r \times 2N$, where r is the number of drug molecules bound per nucleotide computed from the known drug binding parameters, N is the number of nucleotide pairs in a plasmid DNA molecule, and ϕ is the unwinding angle of the drug. In Figure 2, $\bar{\tau}$ is plotted as

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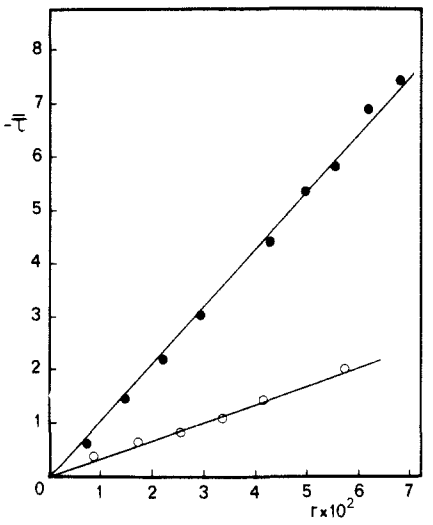

Figure 2. Unwinding of DNA by compounds **22** (●–●) and **23** (○–○). The mean number of superhelical turns ($-\bar{\tau}$) in plasmid DNA samples relaxed by topoisomerase I in the presence of various drug concentrations is plotted vs. the amount of drug molecules bound per nucleotide (r). The unwinding angle (ϕ) of each drug is proportional to the slope of the corresponding line.

a function of r for two different drugs (**22**, **23**). The slopes of the corresponding lines give the values of the unwinding angle, ϕ. As a control, an experiment was performed with 2-methylellipticinium acetate. An unwinding angle of 13° was found. This result is in complete agreement with what is found for this drug (i.e., 14°) by the viscosimetric method.[16] Results obtained for several 11*H*-pyridocarbazoles, in comparison with some 6*H*- and 7*H*-pyridocarbazoles, are given in Table I.

Cytotoxic Activity. The cytotoxicities of compounds **17**–**27** measured on L1210 cells in vitro by growth inhibition or cloning efficiency are quite low compared to 6*H*- and 7*H*-pyridocarbazole analogues[2,5] as shown in Table II.

Discussion. The DNA binding properties of 11*H*-pyridocarbazole derivatives appear to be somewhat different from those observed for 6*H*- and 7*H*-pyridocarbazole derivatives.

11*H*-Pyridocarbazole compounds bind to DNA with binding constants that are close to those observed in the 6*H*- and 7*H*-pyridocarbazole series. However, there is no correlation between these binding constants and intercalation ability as deduced from DNA unwinding and lengthening (ϕ and slope of Table I).

A situation similar to that described by Saucier et al.[15] and by Waring[18] for intercalating compounds is found for 11*H*-pyridocarbazoles. These compounds may be classified into three groups: (a) compounds that clearly intercalate as it can be deduced from the lengthening and the unwinding of the DNA helix (e.g., compounds **20** and **22**. The unwinding angle of compound **22** is small, but similar to that observed for daunorubicin[15]); (b) Compounds that do not intercalate because they do not cause any significant lengthening and unwinding of the DNA helix (e.g., compounds **17**, **23**, and **27**); and (c) compounds that induce too small lengthening and unwinding of the DNA helix to be defined as true intercalators (e.g., compounds **25** and **26**). Saucier et al.[15] and Waring[18] proposed that such compounds may have two simultaneous modes of interaction with DNA: intercalation and outside groove binding. The measurement of the unwinding angle alone is not sufficient to determine the fractions of inside and outside drug bindings. Indeed these authors have observed that non-intercalating compounds, such as irehdiamine A, are able to unwind the DNA helix.[19,20]

Table II. Compared Inhibition Activities of 6*H*-, 7*H*-, and 11*H*-Pyridocarbazoles on L1210 Cell Cultures in Vitro

compd	ED_{50},[a] ng/mL	EC_{37},[b] μg/mL
2-methylellipticinium acetate	544[c]	0.4
2-methyl-9-hydroxyellipticinium acetate	60[c]	0.013
2-methyl-10-methoxy-7*H*-pyrido[4,3-*c*]carbazolium iodide	380[d]	
2-methyl-10-hydroxy-7*H*-pyrido[4,3-*c*]carbazolium iodide	120[d]	
19	>1000	>1
20	>1000	>1
21	>1000	>1
22	>1000	>1
24	>1000	>1
25	>1000	>1
27	>1000	>1

[a] Dose effective in inhibiting 50% of the cell growth after 24-h exposure to the drug. [b] Dose required to inhibit the cloning efficiency to a factor of 0.37 after 24-h exposure to the drug. [c] Unpublished results, courtesy of Dr. S. Cros. [d] Unpublished results.

In the 11*H*-pyridocarbazole series, it appears that the only two unsubstituted analogues **20** and **22** behave as true intercalators. It is then possible to speculate that the introduction of a methoxy or hydroxy group hinders the intercalative binding. Since this situation is not encountered in the 6*H*- and 7*H*-pyridocarbazole series, it is of interest to discuss the mode of DNA binding of these derivatives in relation to their geometry.

The introduction of a methoxy or hydroxy group has minimal effect on the DNA binding properties[5] of the 7*H*-pyridocarbazoles, whereas introduction of these groups leads to a 10-fold increase of the DNA binding constants[2] in the 6*H*-pyridocarbazole series. This enhancement was related to the possible interaction of the hydroxy group on the molecule with the phosphate sugar backbone through hydrogen bonding.

In the case of the 7*H*-pyridocarbazoles, the aromatic ring system is the smallest one among the three pyridocarbazole analogues. Therefore, introduction of a small substituent such as a hydroxy or methoxy group does not hinder the intercalation process. Moreover, the small size of the ring system gives a large freedom of motion in the intercalated site, which might also explain why bisintercalating molecules were more easily obtained in that series:[6] the structural constraint, which appears at the level of the DNA–dimer complex, being more easily compensated for by small displacements of the intercalating ring.

The size and geometry of 6*H*-pyridocarbazoles correspond almost perfectly to that of a DNA base pair as observed by a crystallographic study.[4] From this model, a hydrogen bond involving the hydroxy group in position 9 very likely occurs with the phosphate sugar backbone with almost no freedom of motion for the intercalated ring.

The size of the 11*H*-pyridocarbazoles is somewhat larger than that of the 6*H*-pyridocarbazoles but is still compatible with intercalation of unsubstituted rings as shown for compounds **20** and **22**. However, when the size of these ring systems becomes larger by introduction of a methoxy or hydroxy group, an intercalation process, similar to that seen in a crystal with an oligonucleotide–ellipticine complex,[4] is hindered. Accordingly, the DNA affinity of com-

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Table III. ^{1}H Chemical Shifts and Coupling Constants of 11*H*-Pyridocarbazoles in Me_2SO-d_6, $\delta_{HMDS} = 0$

	proton[a]									
compd	H-1	H-2	H-3	H-4	H-5	H-6	H-7	H-8	H-9	H-10
4		8.87 (4.5)	7.54 (4.5, 8.5)	8.42 (8.5)	7.59 (8.7)	8.26 (8.7)	8.12 (7.5)	7.19 (7.5, 7.0)	7.38 (7.0, 7.0)	7.61 (7.0)
5		8.90 (4.0)	7.55 (4.0, 7.0)	8.36 (7.0)	7.57 (8.0)	8.21 (8.0)	7.69		7.01 (8.5)	7.52 (8.5)
6		8.83 (4.0)	7.46 (4.0, 8.0)	8.34 (8.0)	7.40 (8.0)	8.09 (8.0)	7.49		6.89 (8.5)	7.37 (8.5)
7	8.32 (5.5)	8.57 (5.5)			7.74 (8.5)	8.33 (8.5)	8.19 (7.5)	7.22 (7.5, 7.0)	7.43 (7.0, 8.0)	7.65 (8.0)
8	8.24 (6.0)	8.55 (6.0)		9.28	7.66 (8.0)	8.31 (8.0)	7.71		7.04 (9.0)	7.53 (9.0)
9	8.86 (7.5)	7.58 (7.5, 4.5)	8.86 (4.5)		7.69 (9.0)	8.40 (9.0)	8.16 (8.0)	7.21 (8.0, 7.5)	7.38 (7.5)	7.61 (7.5)
10	8.81 (8.0)	7.56 (8.0, 4.5)	8.83 (4.5)		7.65 (8.5)	8.35 (8.5)	7.72		7.00 (8.5)	7.50 (8.5)
11	8.80 (8.5)	6.88 (8.5, 4.5)	8.82 (4.5)		7.60 (8.5)	8.27 (8.5)	7.44		6.89 (8.5)	7.41 (8.5)
12	9.75		8.50 (6.0)	7.83 (6.0)		8.22	8.07 (9.0)	7.18 (9.0, 9.0)	7.35 (9.0, 8.0)	7.56 (8.0)
13	9.73		8.48 (6.0)	7.82 (6.0)		8.21	7.62		6.97 (8.5)	7.46 (8.5)
14	9.00		8.53 (5.7)	7.90 (5.7)		8.17	7.30		6.90 (8.5)	7.30 (8.5)

	proton										
compd	CH_3	$COCH_3$	OCH_3	CH=C	H-5	H-6	Pyr. β-H	H-4	H-7	H-2	Pyr. α-H
15a	(s) 2.32	(s) 2.67		(m) 7.21	(dd) 7.27	(dd) 7.33	(d) 7.59	(d) 7.81	(d) 8.30	(s) 7.87	(d) 8.51
15b	(s) 2.31	(s) 2.64	(s) 3.78	(m) 7.20		(dd) 6.91	(d) 7.61	(d) 7.61	(d) 8.18	(s) 7.82	(d) 8.52
16a	(s) 2.26			(m) 7.34	(dd) 7.03	(dd) 7.10	(d) 7.52	(d) 7.76	(d) 7.34	(s) 7.58	(d) 8.45
16b	(s) 2.24		(s) 3.75	(m) 7.33		(dd) 6.72	(d) 7.55	(d) 7.27	(d) 7.24	(s) 7.54	(d) 8.45

	proton								
compd	cyclohexane-H	OCH_3	H-8	H-4	H-5	H-6	H-3	H-2	H-1
1	(m) 1.82-3.04	(s) 3.30	(d) 3.67	(d) 7.31	(d) 7.34	(d) 7.56	(dd) 8.23	(d) 8.71	
2	(m) 1.87-3.04	(s) 3.30	(d) 3.69	(d) 9.11	(d) 7.49	(d) 7.64		(d) 8.39	(d) 8.02
3	(m) 1.85-3.04	(s) 3.30	(d) 3.67		(d) 7.46	(d) 7.71	(d) 8.64	(dd) 7.40	(d) 8.59

[a] *J* coupling constants (Hz) are in parentheses.

pound **27** is quite high and intercalation is not observed. Probably, this compound prefers to lie on the external of the DNA helix as do many other aromatic compounds.[21]

The cytotoxicity on L1210 cells in vitro of the 7*H*-pyridocarbazoles appears in the same range as that of the antitumor agent 2-methyl-9-hydroxyellipticinium acetate, which belongs to the 6*H*-pyridocarbazole series (Table II). In contrast, none of the 11*H*-pyridocarbazoles elicits a measurable cytotoxicity. Such a behavior cannot be accounted for by differences in the DNA binding properties alone. It has recently been proposed[22-26] that intercalating drugs could act by interfering with the DNA topoisomerase II action. Because some ellipticine derivatives[27,28] were shown to interact with DNA topoisomerase II action, a comparative study of the three pyridocarbazole analogues with this enzyme would be warranted.

Experimental Section

Melting points were determined on a Kofler apparatus and are not corrected. For NOE experiments, 10^{-2} M deuteriated dimethyl sulfoxide solutions of samples were filtered and degassed by bubbling helium. ^{1}H NMR spectra were recorded at 270 MHz on a WH 270 Brucker spectrometer equipped with a Brucker temperature controller unit. Data were treated with an Aspect 2000 computer. Resolution enhancements were achieved by use of an exponential multiplication of the fid. Chemical shifts are given in ppm ± 0.01 ppm from hexamethyldisiloxane as internal reference. UV spectra and molar extinction coefficients were recorded on a Beckman Acta III spectrophotometer. Fluorometric measurements were conducted with a photon-counting SLM-800 (Urbana, IL) or with a Kontron SFM 23/B (Zurich, Switzerland) spectrofluorometer. Purification was achieved by silica gel preparative layer chromatography (chloroform/methanol, 93:7), or by semipreparative HPLC on an ultrasphere ODS column (Altex) (methanol/0.1 M ammonium acetate, pH 6, 70:30). Sample purity was checked by HPLC on a μBondapack C_{18} column (Waters Assoc.) as already described.[29] Sonicated calf thymus DNA was prepared as described previously.[15] Analyses indicated only by symbols of the elements mean that analytical results obtained for these elements were within ±0.4 of the theoretical values.

8-Methoxy-7,8,9,10-tetrahydro-11*H*-pyrido[2,3-*a*]carbazole (1). 8-Quinolylhydrazine dihydrochloride[30] (5 g, 20 mmol) and sodium acetate (5 g) dissolved in ethanol/water (35 mL/20 mL) were treated with 4-methoxycyclohexanone (2.5 g, 25 mmol) at reflux for 2.5 h. The solvents were evaporated under reduced pressure, and the crude hydrazone thus obtained was refluxed with acetic acid/concentrated sulfuric acid (30 mL/4 mL) for 15 min. After cooling, the mixture was poured in ice water with vigourous shaking and basified with ammonium hydroxyde then extracted with dichloromethane. The organic layer was dried over sodium sulfate and the solvent evaporated under reduced pressure. The residue gave gray-beige crystals, which were collected by filtration. After drying in vacuo, 2.7 g (49.7%) of material was obtained: mp 146 °C. Anal. ($C_{16}H_{16}N_2O$) C, H, N, O.

8-Methoxy-7,8,9,10-tetrahydro-11*H*-pyrido[4,3-*a*]carbazole (2). Following the above procedure, 5-isoquinolylhydrazine dihydrochloride[30] (5 g, 20 mmol) and 4-methoxycyclohexanone (2.5 g, 25 mmol) afforded 2.4 g (44%) of 8-methoxy-7,8,9,10-tetrahydro-11*H*-pyrido[4,3-*a*]carbazole: mp 183 °C. Anal. ($C_{16}H_{16}N_2O$) C, H, N, O.

8-Methoxy-7,8,9,10-tetrahydro-11*H*-pyrido[3,2-*a*]carbazole (3). The same procedure as above with 5-quinolylhydrazine dihydrochloride[30] (5 g, 20 mmol) and 4-methoxycyclohexanone (2.5 g, 25 mmol) gave 2.3 g (42.3%) of material: mp 254 °C. Anal. ($C_{16}H_{16}N_2O$) C, H, N, O.

8-Methoxy-11*H*-pyrido[2,3-*a*]carbazole (5). 8-Methoxy-7,8,9,10-tetrahydro-11*H*-pyrido[2,3-*a*]carbazole (1) (300 mg) and 10% palladium on activated charcoal (600 mg) were refluxed in decahydronaphthalene (100 mL) for 6 h. The solvent was filtered while still hot and, on cooling, **5** crystallized as colorless crystals. After filtration with suction followed by several washings with

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40–65 °C petroleum ether, the precipitate was dried in vacuo affording 107 mg, which was purified by semipreparative HPLC giving 60 mg (20.3%) of analytically pure material: mp >270 °C. Anal. ($C_{16}H_{12}N_2O\cdot0.75H_2O$) C, H, N.

Compound **5** (3 mmol) treated in 2 mL of methanol with ether saturated with hydrogen chloride afforded 28 mg of the pyridocarbazolium chloride **18**: mp >270 °C. Anal. ($C_{16}H_{13}ClN_2O$) C, H, N.

Similarly, 11*H*-pyrido[2,3-*a*]carbazole **4**[8,10] afforded 34 mg of hydrochloride **17**: mp >270 °C. Anal. ($C_{15}H_{11}ClN_2\cdot CH_3OH$) C, H, N.

8-Methoxy-11*H*-pyrido[4,3-*a*]carbazole (8). 8-Methoxy-7,8,9,10-tetrahydro-11*H*-pyrido[4,3-*a*]carbazole (**2**) (300 mg) and 10% palladium on activated charcoal were treated as described for **5** affording 103 mg. After purification by semipreparative HPLC, 45 mg (15.3%) of analytically pure material was obtained: mp >270 °C. Anal. ($C_{16}H_{12}N_2O$) C, H, N.

Compound **8** (2 mmol) was dissolved in 3 mL of dry dimethylformamide at 40 °C, and 4 μL of methyl iodide was added. After stirring overnight, the methiodide **21** precipitated (28 mg): mp >270 °C. Anal. ($C_{17}H_{15}IN_2O$) C, H, N.

Similarly, 11*H*-pyrido[4,3-*a*]carbazole **7**[7] afforded 31 mg of **20**: mp >270 °C. Anal. ($C_{16}H_{13}IN_2\cdot0.5H_2O$) C, H.

8-Methoxy-11*H*-pyrido[3,2-*a*]carbazole (10). As described for **5**, 8-methoxy-7,8,9,10-tetrahydro-11*H*-pyrido[3,2-*a*]carbazole **3** (300 mg) afforded 42 mg (14.3%) of analytically pure **10** after purification by semipreparative HPLC: mp >270 °C. Anal. ($C_{16}H_{12}NO$) C, H, N.

Compound **10** gave 30 mg of methiodide **23**: mp >270 °C. Anal. ($C_{17}H_{15}IN_2O\cdot H_2O$) C, H, N.

Similarly, 11*H*-pyrido[3,2-*a*]carbazole **9**[8,10] afforded 30 mg of methiodide **22**: mp >270 °C. Anal. ($C_{16}H_{13}IN_2\cdot2H_2O$) C, H, N.

1-(1-Acetylindol-3-yl)-2-(4-pyridyl)prop-1-ene (15a). A mixture of 3-formylindole (3 g, 20.7 mmol) and 4-ethylpyridine (10 g, 93 mmol) in acetic anhydride (100 mL) was refluxed for 18 h. Approximately two-thirds of the solvent was evaporated off, and the residue was treated with 3 N hydrochloric acid (40 mL). The precipitate was filtered off and partitioned between aqueous potassium carbonate and dichloromethane, and the dried organic phase was evaporated to give the product, which crystallized in ethanol affording 1.4 g (24.6%) of material: mp 140 °C. Anal. ($C_{18}H_{16}N_2O$) C, H, N, O.

1-(5-Methoxy-1-acetylindol-3-yl)-2-(4-pyridyl)prop-1-ene (15b). As described for **15a**, 3 g of 5-methoxy-3-formylindole afforded 1.1 g (21%) of material after crystallization in ethanol: mp 158 °C. Anal. ($C_{19}H_{18}N_2O_2$) C, H, N, O.

1-(Indol-3-yl)-2-(4-pyridyl)prop-1-ene (16a). The *N*-acetyl compound **15a** (1 g) in ethanol (50 mL) was treated with a 25% solution of sodium hydroxide for 15 min at room temperature. The solution was concentrated, diluted with water, and extracted with dichloromethane. The dried organic solvent was evaporated to give 800 mg (94%) of material, which was used without further purification for the next step: mp 220 °C. Anal. ($C_{16}H_{14}N_2$) C, H, N.

1-(5-Methoxyindol-3-yl)-2-(4-pyridyl)prop-1-ene (16b). As described for **16a**, 1 g of **15b** gave 776 mg (90%) of **16b**: mp 199 °C. Anal. ($C_{17}H_{16}N_2O$) C, H, N, O.

5-Methyl-11*H*-pyrido[3,4-*a*]carbazole (12). Compound **16a** (600 mg) in 95% ethanol (1000 mL) was irradiated (100-W Hanovia medium pressure lamp, Pyrex filter) for 7 h with stirring. The solvent was evaporated to dryness and the residue worked up with hexane. After filtration and drying in vacuo, the 400 mg obtained was purified on silica gel preparative layer chromatography plates (chloroform/methanol, 93:7) affording 85 mg (14.2%) of analytically pure material: mp >270 °C. Anal. ($C_{16}H_{12}N_2\cdot2H_2O$) C, H, N.

Four microliters of methyl iodide was added to **12** (2 mmol) in 3 mL of dry dimethylformamide at 40 °C. After stirring overnight, the methiodide **25** precipitated (25 mg): mp >270 °C. Anal. ($C_{17}H_{15}IN_2\cdot1.5H_2O$) C, H, N.

5-Methyl-8-methoxy-11*H*-pyrido[3,4-*a*]carbazole (13). Following the procedure described for **12**, compound **15b** (800 mg) gave 87 mg (11%) of pure material: mp >270 °C. Anal. ($C_{17}H_{14}N_2O\cdot H_2O$) C, H, N.

Compound **13** (2 mmol) gave 29 mg of methiodide **26**: mp >270 °C. Anal. ($C_{18}H_{17}IN_2O\cdot0.5H_2O$) C, H, N.

8-Hydroxy-11*H*-pyrido[2,3-*a*]carbazole (6). 8-Methoxy-11*H*-pyrido[2,3-*a*]carbazole **5** (60 mg, 0.24 mmol) in 1.5 mL of 48% aqueous hydrobromic acid was stirred for 5 h at 120 °C. The solution was then cooled down to 40 °C, poured into 40 mL of water, and treated with sodium carbonate until no more precipitation occurred, final pH 9. After filtration and drying, **6** was purified on silica gel preparative layer chromatography plates (chloroform/methanol, 93:7) affording 22 mg (38.8%) of pure material: mp >270 °C. Anal. ($C_{15}H_{10}N_2O$) C, H, N.

Compound **6** (3 mmol) in 2 mL of methanol treated with ether saturated with hydrogen chloride afforded 29 mg of pyridocarbazolium chloride **19**: mp >270 °C. Anal. ($C_{15}H_{11}ClN_2O$) C, H, N.

8-Hydroxy-11*H*-pyrido[3,2-*a*]carbazole (11). As for **6**, 8-methoxy-11*H*-pyrido[3,2-*a*]carbazole **10** (40 mg, 0.16 mmol) afforded 15 mg (39.5%) of pure **11**: mp >270 °C. Anal. ($C_{15}H_{10}N_2O$) C, H, N.

Four microliters of methyl iodide was added to **11** (2 mmol) in 3 mL of dry dimethylformamide at 40 °C, yielding methiodide **24** (31 mg) after stirring overnight: mp >270 °C. Anal. ($C_{16}H_{13}IN_2O\cdot1.5H_2O$) C, H, N.

5-Methyl-8-hydroxy-11*H*-pyrido[3,4-*a*]carbazole (14). Following the procedure described for **6**, compound **13** (60 mg, 0.24 mmol) afforded 15 mg (26.5%) of pure material: mp >270 °C. Anal. ($C_{16}H_{12}N_2O\cdot H_2O$) C, H, N.

Compound **14** (2 mmol) gave 26 mg of methiodide **27**: mp >270 °C. Anal. ($C_{17}H_{15}IN_2O\cdot3H_2O$) C, H, N.

Interaction with DNA. The K_{ap} values were determined at 25 °C in 0.2 M sodium acetate buffer, pH 5.0, by fluorescence measurements with excitation at 540 nm and emission at 610 nm. Four cells were used simultaneously for each experiment. The first cell contained the buffer, the second one the DNA (1.5 μg/mL), and the third and fourth ones the drug (3 and 6 μg/mL, respectively) plus DNA. Microliter aliquots of ethidium bromide (50 μg/mL of buffer) were added. Scatchard plots of competition were calculated with a 9810-1 Hewlett-Packard calculator.

To measure the length increase of short DNA segments, the intrinsic viscosity of sonicated calf thymus DNA (100 μg/mL) in the presence of increasing concentrations of drug (from 0 to 10 μg/mL) was measured at 25 °C in a semimicro dilution capillary viscosimeter (Cannon Instrument Co., State College, PA). Flow times were measured to ±0.1 ms by the combined use of photoelectric sensors and an electronic timer.[31]

The unwinding angle of supercoiled DNA is measured by relaxation of plasmid pUC13 DNA (1 μg) with rat liver topoisomerase I, in the presence of increasing drug concentrations (from 1 to 10 μg/mL). The relaxation is performed at 37 °C in 0.2 M sodium chloride and 20 mM sodium acetate buffer, pH 5. Samples are treated as described by Keller.[17]

Drug Exposure and Cell Survival Determination. Exponentially growing L1210 cells (1×10^5/mL) were incubated for 24 h at 37 °C in RPMI 1640 supplemented with fetal calf serum (10%) medium containing different drug concentrations.[32] Total cell number in the different cultures was determined by means of a Cytograf, Model 6300 A (Bio/Physics Systems, Inc.). The average number of cells in each duplicate treated cultures was expressed as a percentage of the average number of cells in the triplicate untreated controls. The ED_{50} value was obtained by plotting the percentage of residual cells vs. the drug concentration on a semilogarithmic scale. The ED_{50} was determined from a linear regression.

For the determination of the cloning efficiency,[32] the cells treated for 24 h were diluted in RPMI 1640 to concentrations of 250 and 500 cells/mL; 2.5 mL of suspension was then added to 0.4 mL of Noble agar (Difco Laboratories) solution before plating on 35-mm-diameter Petri dishes. After 14 days of incubation at 37 °C in an atmosphere of 5% carbon dioxide humidified air, colonies larger than 0.2 mm in diameter were counted by means of a Biotran III counter (New Brunswick Co., Inc.). Triplicate assays were carried out at each drug concentration. The cloning efficiency in the controls was about 65%. Dose–response curves

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were used to determine the mean lethal concentration (CE_{37}), defined as the concentration required to reduce cloning efficiency to a factor of 0.37.

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Registry No. 1, 102852-77-7; 2, 102852-78-8; 3, 102852-79-9; 4, 239-13-4; 5, 102852-59-5; 6, 102852-60-8; 7, 239-03-2; 8, 102852-61-9; 9, 239-06-5; 10, 102852-62-0; 11, 102852-63-1; 12, 53645-54-8; 13, 102852-64-2; 14, 102852-65-3; 15a, 102852-80-2; 15b, 102852-81-3; 16a, 76402-00-1; 16b, 102852-82-4; 17, 102852-66-4; 18, 102852-67-5; 19, 102852-68-6; 20, 102852-69-7; 21, 102852-70-0; 22, 102852-71-1; 23, 102852-72-2; 24, 102852-73-3; 25, 102852-74-4; 26, 102852-75-5; 27, 102852-76-6; 8-quinolylhydrazine dihydrochloride, 91004-61-4; 4-methoxycyclohexanone, 13482-23-0; 4-methoxycyclohexanone 8-quinolylhydrazone, 102852-55-1; 5-isoquinolylhydrazine dihydrochloride, 102852-56-2; 4-methoxycyclohexanone 5-isoquinolylhydrazone, 102852-57-3; 5-quinolylhydrazine dihydrochloride, 91004-60-3; 4-methoxycyclohexanone 5-quinolylhydrazone, 102852-58-4; 3-formylindole, 487-89-8; 4-ethylpyridine, 536-75-4; 5-methoxy-3-formylindole, 10601-19-1; 2-methylellipticinium acetate, 69492-82-6; 2-methyl-9-hydroxyellepticinium acetate, 58337-35-2; 2-methyl-10-methoxy-7*H*-pyrido[4,3-*c*]carbazolium iodide, 62099-82-5; 2-methyl-10-hydroxy-7*H*-pyrido[4,3-*c*]carabazolium iodide, 62099-85-8.

Comparative Toxicities and Analgesic Activities of Three Monomethylated Analogues of Acetaminophen

Peter J. Harvison, Anthony J. Forte, and Sidney D. Nelson*

Department of Medicinal Chemistry, School of Pharmacy, University of Washington, BG-20, Seattle, Washington 98195.
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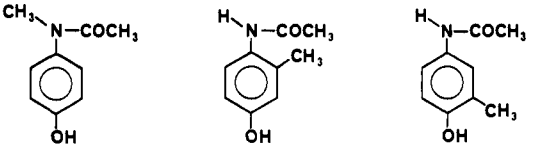
Three monomethylated derivatives of 4′-hydroxyacetanilide (acetaminophen) were prepared in order to compare their cytotoxic potential and analgesic activity with that of acetaminophen. Only 4′-hydroxy-*N*-methylacetanilide (*N*-methylacetaminophen) was devoid of cytotoxic effects to hepatic tissue of mice. Results of comparative tissue distribution studies and metabolism studies both in vivo and in vitro in mice indicate that the disposition of *N*-methylacetaminophen is similar to that of acetaminophen except that it is not oxidized to a toxic metabolite. In contrast, 3′-methyl-4′-hydroxyacetanilide (3-methylacetaminophen) is as hepatotoxic as acetaminophen in mice while 2′-methyl-4′-hydroxyacetanilide (2-methylacetaminophen) is less hepatotoxic. The analgesic potency of the analogues seems to parallel their hepatotoxic potential, and both activities parallel the oxidation potentials in this series of compounds.

The widely used analgesic drug, acetaminophen (1), is known to cause serious liver necrosis at high doses in man and experimental animals.[1] An electrophilic product of cytochrome P-450 oxidation that depletes cellular glutathione (GSH) and covalently binds to tissue macromolecules has been implicated in this toxic reaction.[2] Initially, the formation of the arylating metabolite was believed to involve N-oxidation of acetaminophen to *N*-hydroxyacetaminophen (3) followed by dehydration to *N*-acetyl-*p*-benzoquinone imine (NAPQI, 2, Figure 1).[3] *N*-Hydroxyacetaminophen was synthesized in crystalline form and found to possess both chemical and toxicological characteristics that were consistent with its postulated role as an intermediate in the metabolism of acetaminophen to an arylating metabolite.[4] However, subsequent studies have shown that if *N*-hydroxyacetaminophen is an intermediate in microsomal oxygenase-mediated covalent binding of acetaminophen to tissue protein, it must decompose at the enzymatic site of hydroxylation.[5] NAPQI, on the other hand, has been recently detected as a cytochrome P-450 catalyzed oxidation product of acetaminophen in reactions that utilize cumene hydroperoxide, and NAPQI has been found to be highly cytotoxic.[6]

Inasmuch as N-oxidation and attack by tissue nucleophiles on the oxidized aromatic ring of acetaminophen are features that correlate with hepatotoxicity, we prepared the three monomethylated analogues (4–6) with the expectation that N-oxidation and/or reaction with tissue nucleohpiles would be hindered. We have previously documented that the *N*-methyl analogue of acetaminophen was not hepatotoxic or nephrotoxic in animal models for these toxic reactions caused by acetaminophen.[7] We now



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